IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 08-350-WO-US)

In application of	)	
	)	
Lorensten, *et al.*	)	**Examiner: Sheridan Swope**
	)	
Serial No. 10/553,869	)	
	)	**Group Art Unit: 1652**
Filed: October 21, 2005	)	
	)	
For: Cleavage of Fusion Protein Using Granzyme B Protease	)	**Confirmation No.: 1881**

APPLICANTS' APPEAL BRIEF

This Appeal Brief is submitted in accordance with the requirements of 37 C.F.R 41.37 and is filed in furtherance of the Notice of Appeal filed December 14, 2010.

The required fee associated with this Appeal Brief according to 37 C.F.R. § 41.20 (b)(2) of $540 has not been paid because Applicants have previously paid the fee for their Appeal Brief filed March 3, 2010. The Examiner withdrew the Appeal and re-opened prosecution with the Office Action of June 14, 2010. Therefore, Applicants understand that no fee is due for this Appeal Brief.

Please charge any additional fees or credit any overpayments to Deposit Account No. 132490.

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I. REAL PARTY IN INTEREST

The real parties in interest are Anaphore, Inc. and Hoffman-La Roche Inc., the assignees of record.

I. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

II. STATUS OF CLAIMS

Claims 1, 4, 6, 8-41, and 43-51 are pending.

Claims 12 and 18-39 were withdrawn from further consideration pursuant to 37 CFR 1.142(b).

Claims 2, 3, 5, 7 and 42 are canceled.

Claims 1, 4, 6, 8-11, 13-17, 40, 41, and 43-51 are under prosecution and stand rejected and objected to.

Applicants appeal the rejections and objection to claims 1, 4, 6, 8-11, 13-17, 40, 41 and 43-51.

III. STATUS OF AMENDMENTS

Applicants filed an amendment after filing the Notice of Appeal on January 28, 2011. The Amendment was entered in the Advisory Action mailed February 10, 2011.

IV. SUMMARY OF CLAIMED SUBJECT MATTER

Independent claims 1 and 40 are generally drawn to a method for preparing a polypeptide of interest in authentic form by enzymatic cleavage of fusion proteins. The method comprises a step of providing a fusion protein comprising from its N-terminal to its C-terminal, a fusion

partner, a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site, and a polypeptide of interest, wherein the cleavage site is placed adjacent to the polypeptide of interest. The fusion protein is subsequently contacted with Granzyme B protease to cleave the fusion protein at the Granzyme B protease cleavage site to yield the polypeptide of interest in authentic form. *E.g.,* Specification p. 6, ll. 12-20.

Independent claim 1

The Granzyme B protease recognition site of claim 1 has the general formula "P4 P3 P2 P1 ↓" wherein P4 is amino acid I or V, P3 is amino acid E, Q, or M, P2 is X, wherein X denotes any amino acid, P1 is amino acid D, and ↓ is the Granzyme B cleavage site. *E.g.,* Specification, p. 8, ll. 21-25. In the method of the invention, the cleavage site is located adjacent the polypeptide of interest. *E.g.,* Specification, p. 7, ll. 8-24.

Granzyme B proteases are granule-stored serine proteases and include enzymes which are or may be classified under the Enzyme Commission number EC 3.4.21.79. *E.g.,* Specification p. 7, ll. 26-32.

Any suitable Granzyme B protease may be used in accordance with the invention including human Granzyme B protease, mouse Granzyme B protease and rat Granzyme B protease. Specification, p. 12, lns. 20-22.

A "fusion partner" is a peptide, oligopeptide, polypeptide or protein; *e.g.,* an affinity tag for supporting isolation of an expressed polypeptide. *E.g.,* Specification, p. 12, ll. 7-19, p. 13, ll. 20-26.

A "polypeptide of interest" is a polypeptide for which expression is desired. The specification describes a broad range of "polypeptides of interest." *E.g.,* Specification, p. 11, lns. 4-23.

A "polypeptide of interest in authentic form" refers to a polypeptide which comprises the amino acid sequence thereof without any additional amino acid residues. To put it another way, in the present context the polypeptide of interest in authentic form refers to a polypeptide having the same primary amino acid sequence as that encoded by the gene sequence coding for the polypeptide of interest, *i.e.* it does not contain any non-native amino acids. Specification p. 6, ln. 24 – p .7, ln. 2.

Independent Claim 40

Independent claim 40 is similar to claim 1, with the primary difference being that claim 40 recites a number of specific Granzyme B cleavage sites. *E.g.,* Specification, p. 10, ln. 29 – p. 11, ln. 4

V. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The rejections on appeal are as follows:

(1) Claims 1, 9-11, 16, 17, 40, 44-46, 50, and 51 are rejected under 35 U.S.C. 103(a) as obvious over Azad, *et al.* in view of Harris, et al, and further in view of Casciola-Rosen, *et al.*

(2) Claims 1, 4-6, 9-11, 16, 17, 40, 41, 44-46, 50, and 51 are rejected under 35 U.S.C. 103(a) as obvious over Azad, *et al.*, Harris, *et al.*, and Casciola-Rosen, *et al.* in view of Boutin, *et al.*

(3) Claims 1, 9-11, 13-17, 40, and 44-51 are rejected under 35 U.S.C. 103(a) as obvious over Azad, *et al.*, Harris, *et al.*, and Casciola-Rosen, *et al.*, in view of Sigma Inc. 1998 or Pharmacia, Inc.

(4) Claims 8 and 43 are rejected under 35 U.S.C. § 103(a) as obvious over Wan, *et al.,* in view of Bleackley, *et al.,* and further in view of Harris, *et al.*

VI. ARGUMENT

A. Rejection of claims 1, 9-11, 16, 17, 40, 44-46, 50, and 51 under 35 U.S.C. 103(a) as obvious over Azad, *et al.* in view of Harris, et al, and further in view of Casciola-Rosen, *et al.*

Claims 1, 9-11, 16, 17, 40, 44-46, 50, and 51 stand rejected under 35 U.S.C. 103(a) as obvious over Azad, *et al.* in view of Harris, *et al.*, and further in view of Casciola-Rosen, *et al.* because the Examiner alleges that it would have been obvious to a person of ordinary skill in the art to modify the fusion protein of Azad, *et al.* to incorporate the motif IEAD, as taught by Harris, *et al.* (FIG. 5D), between the GST fusion partner and nef27, and then generate nef27 by cleaving the fusion protein with Granzyme B protease. The Examiner alleges that the motivation to combine Azad, *et al.* and Harris, *et al.* derives from the desire to produce nef27. The Examiner further alleges, although incorrectly, that Applicants acknowledge that it would have been obvious to cleave a fusion protein with Granzyme B, as was known in the art (referring to Casciola-Rosen, *et al.*).

The Examiner has improperly rejected claims 1, 9-11, 16, 17, 40, 44-46, 50, and 51 under 35 U.S.C. 103(a) as obvious over Azad, *et al.* in view of Harris, *et a.l,* and further in view of Casciola-Rosen, *et al.* for a number of reasons.

As a threshold matter, a claimed invention is unpatentable if the differences between it and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art." 35 U.S.C. § 103(a); *see Graham v. John Deere Co.*, 383 U.S. 1, 14 (1966). The ultimate determination of whether an invention is or is not obvious is based on underlying factual inquiries including: (1) determining the scope and content of the prior art; (2) ascertaining the differences between the prior art and

the claims at issue; (3) resolving the level of ordinary skill in the pertinent art; and (4) evaluating evidence of secondary considerations. *See Graham, 383 U.S. at 17-18.*

The Supreme Court emphasizes that the key of supporting any rejection under 35 U.S.C. §103 is the clear articulation of the reason(s) why the claimed invention would have been obvious. *KSR Int'l Co. v. Teleflex Inc.,* 127 U.S. 1727, 1741 (2007). The Court, quoting *In re Kahn*, stated that "rejections on obviousness cannot be sustained with mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *Id., citing, In re Kahn*, 441, F.3d 977, 988 (Fed. Cir. 2006).

At the outset, it should be appreciated that instant claims are directed to a method for the preparation of a polypeptide of interest in authentic form. In contrast, this feature of the instant claims is not taught or suggested in any of Azad, *et al.*, or Harris, *et al.*, or Casciola-Rosen, *et al.* The failure of asserted references to teach or suggest each and every feature of instant claims is fatal to an obviousness rejection under 35 U.S.C. § 103. Section 2143.03 of the MPEP requires the "consideration" of every claim feature in an obviousness determination. To render instant claims unpatentable, however, the Office must do more than merely "consider" each and every feature for this claim. Instead, the asserted references, individually or in combination, even if supported by the motivation to combine, must also teach or suggest each and every claim feature. *See In re Royka,* 490 F.2d 981, 180 USPQ 580 (CCPA 1974) (to establish *prima facie* obviousness of a claimed invention, all the claim features must be taught or suggested by the prior art).

In a recent case, the Federal Circuit reiterated that in order to support a conclusion of obviousness, the combined prior art must teach all of the elements of the claimed invention.

Honeywell Int'l Inc. v. United States, 609 F.3d 1292, 95 U.S.P.Q.2d 1193 (Fed. Cir. 2010). *See also In re Wada and Murphy*, Appeal 2007-3733, *citing In re Ochiai,* 71 F.3d 1565, 1572 (Fed. Cir. 1995) (a proper obviousness determination requires that an Examiner make "a searching comparison of the claimed invention – *including all its limitations* – with the teaching of the prior art." (emphasis in original)).

Further, the necessary presence of all claim features is axiomatic, since the Supreme Court has long held that obviousness is a question of law based on underlying factual inquiries, including … ascertaining the differences between *the claimed invention* and the prior art. *Graham v. John Deere Co.,* 383 U.S. 1, 148 USPQ 459 (1966). MPEP § 2143 requires that the prior art provide at least a suggestion of all of the features of a claim in the prior art. This suggestion should serve as the foundation of an "articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR Int'l v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) (*quoting In re Kahn,* 441 F.3d 977, 988 (Fed. Cir. 2006).

(i) None of the cited references teach or suggest the production of a polypeptide in authentic form

In the Office Action mailed June 14, 2010, the Examiner sets forth the following fictitious examples of polypeptides that are described as "the fusion proteins to be used in the methods rendered obvious by the combination of Azad, et al, Harris, et al., and Casciola-Rosen, et al:"

(i) GST-IEAD↓[N-met-Gly-nef27-C]

(ii) HIS_{6X}-IEAD↓[N-met-Gly-nef27-C]

wherein GST and HIS_{6X} a fusion partners, [N-met-Gly-nef27-C] is the authentic sequence for nef27, IEAD is a cleavage motif for Granzyme B, and ↓ indicates the cleavage position for Granzyme B. *See* Office Action, p. 5.

These examples of fusion proteins are simply the creativity of the Examiner, and not found anywhere in the prior art. Also, the Examiner is not correct in describing [N-met-Gly-nef27-C] as the "authentic sequence for nef27." Indeed, the Office Action even acknowledges that Azad, et al, which the Examiner uses for the reference to nef27, "does not teach the production of a polypeptide of interest in authentic form." Office Action ,p. 8, last paragraph.

While acknowledging the deficiency in the prior art to teach this essential feature of the claims, the Examiner asserts that it is the combination of the references that renders the claimed invention obvious. The Examiner's conclusion, however, is completely inconsistent with the Examiner's acknowledgement that the prior art does not teach "the production of a polypeptide of interest in authentic form." *Id.* Simply put, the combination of prior art cannot render the invention obvious if the prior art does not teach all of the elements of the claims. *Honeywell Int'l Inc. v. United States,* 609 F.3d 1292; *In re Wada and Murphy*, Appeal 2007-3733; *In re Ochiai,* 71 F.3d at 1572; *In re Royka,* 499 F.2d at 981.

Not only does the prior art failed to teach the production of a polypeptide of interest in authentic form, the art also fails to teach a polypeptide of interest in authentic form adjacent the Granzyme B cleavage site as recited in independent claims 1 and 40. The definition of authentic form in the specification is clear: "authentic form" refers to a polypeptide that has "no extraneous amino acids derived from the cleavage site." Specification, p. 5, ll. 5-7. Therefore, the polypeptide of interest in authentic form refers to a polypeptide having the same primary amino acid sequence as that encoded by the gene sequence coding for the polypeptide of interest;

i.e., it does not contain any non-native amino acids. *Id.,* p. 6, ln. 24 - p.7, ln. 2. As a point of clarification, the specification also describes that in the claimed invention, a polypeptide of interest in authentic form is not always a polypeptide that occurs in nature, but it may also be partially or completely artificial. Specification p. 7, ll. 3-7.

There is no dispute that Harris, *et al.* does not teach that a polypeptide of interest in authentic form is adjacent the Granzyme B cleavage site. Figure 5 and the remainder of Harris, *et al.* teach the cleavage of a fusion protein to produce a pIII coat protein of M13 bacteriophage. Harris, *et al.* discloses a six amino acid motif – *e.g.,* IEAD↓AL -- that is explained as essential for Granzyme B cleavage (Abstract and Figure 5). The amino acids following the cleavage site, the P1' and P2' amino acids and a linker (AGPGGG), are not part of the authentic polypeptide sequence of the pIII coat protein of M13 bacteriophage, which is the polypeptide of interest in Harris, *et al.* *See* p. 27365, last paragraph of col. 2. Therefore, following cleavage at the cleavage site (↓), the polypeptide of interest is left with two non-authentic peptides (AL) at the N-terminus.

Also, Azad, *et al.* does not teach a polypeptide of interest in authentic form adjacent a cleavage site. In earlier Office Actions, the examiner refers to p. 651, ¶ 2 of Azad for as teaching that a nef27 polypeptide contains Met-Gly at the N-terminus. *See* Office Action mailed April 7, 2009, p. 6 and Advisory Action mailed June 18,2009. However, Azad, *et al.* teaches the production of the nef27 protein using the pGEX-2T fusion vector described in Azad, *et al.* (See p. 651, last paragraph). This vector includes a thrombin recognition sequence and cleavage site in the GST peptide encoded by the vector: Leu-Val-Pro-Arg↓Gly-Ser, wherein "↓" is the thrombin cleavage site. *See* Ex. A (Evidence Appendix) which is a map of the pGEX-2T vector showing the cleavage site. Therefore, the Nef protein derived from thrombin-cleaved GST-Nef

(see p. 653) is left with Gly-Ser from the vector at the N-terminus. Because the Nef peptide produced as described in Azad, *et al.* has non-native amino acids left over from the vector (Gly-Ser) at the N-terminus, the authentic nef27 in Azad, *et al.* is not adjacent the cleavage site and Azad, *et al.* does not teach the production of a polypeptide in authentic form as presently claimed.

In the recent Office Action, the Examiner states that the "teaching of Azad et al regarding the Leu-Val-Pro-Arg↓Gly-Ser motif and cleavage of thrombin are not used for the instant rejection. *See* Office Action mailed June 14, 2010, p. 9. Instead, the Examiner states that the "relevant teaching of Azad et al is the generic idea of cleaving a fusion protein comprising nef27 to release nef27 protein." *Id.* But as addressed above, the nef27 is not in authentic form as presently claimed. If indeed the only relevant teaching from Azad, *et al.* is a generic teaching of cleaving a fusion protein to produce a non-authentic polypeptide, then Azad, *et al.* is merely cumulative of Harris, *et al.*

With regard to Casciola-Rosen, *et al.,* this reference teaches a number of Granzyme B cleavage motifs, but it does not teach cleavage of fusion proteins or the production of a polypeptide in authentic form.

Therefore, none of Harris, *et al.,* Azad, *et al.,* or Casciola-Rosen, *et al.* teach a polypeptide of interest in authentic form adjacent a Granzyme B cleavage site or the production of a polypeptide in authentic form.

(ii) Because none of the references teach or suggest the production of the authentic form of polypeptide of interest, the reference can not be combined to render obvious the present invention.

Harris et al. provides no reason for one of ordinary skill in the art to use its method to produce a polypeptide in authentic form as presently being claimed. Indeed, Harris, *et al.*

teaches away from the present invention because Harris, *et al.* teaches the necessity of P1' and P2' amino acids (amino acids that are in the C-terminal direction from the cleavage site).

Furthermore, instead of teaching or suggesting the production of a polypeptide of interest in authentic form, Harris, *et al.* describes the cleavage of a variety of short synthetic amide substrates produced via a combinatorial library as shown in Tables 2 and 3. Harris, *et al.* merely identifies a handful of six amino acid sequences and the specific site of Granzyme B cleavage and provides no mention or suggestion to use Granzyme B for the purification of protein of interest in authentic form. To put it another way using the words of the CAFC in *In re O' Farrell*, Harris *et al.* gives one skilled in the art "no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful" to arrive at the claimed invention. *In re O'Farrell*, 853 F.2d 894, 895 (Fed. Cir. 1988).

Moreover, while Casciola-Rosen, *et al.* teaches a number of Granzyme B cleavage motifs, it does not teach cleavage of fusion proteins or the production of a polypeptide in authentic form. Thus, Casciola-Rosen, *et al.* does not cure the deficiencies of Harris, *et al.* and Azad, *et al.*

The Examiner has not suggested any motivation to combine any of the references except for the general overall motivation to produce polypeptides in authentic form. While Applicants do not dispute that there is a motivation for protein chemists to make pure, authentic polypeptide, the Examiner has not cited any rational reasoning why a skilled artisan would choose the method of Harris, *et al.,* which the Examiner agrees does not teach the production of a polypeptide of interest in authentic form, to produce a nef27 polypeptide of Azad, *et al.*. The Examiner states that it would have been obvious to adapt the fusion protein to replace the GST fusion partner with an 6X-His fusion partner of Harris, *et al.*. Even doing so, however, does not render the

polypeptide of interest in authentic form because, as explained above, the cleave motif of Harris does not product a polypeptide of interest in authentic form.

As recently reiterated in Bayer *Schering Pharma AG v. Barr Laboratories Inc.*, 91 USPQ2d 1569, 1573 (Fed. Cir. 2009), generalities or vague or non-existent guidance towards the claimed invention is insufficient to render a claim obvious; there must be some reason for the ordinary artisan to make the particular invention being claimed. Harris, *et al.* provides no reason for one of ordinary skill in the art to use its method to produce a polypeptide in authentic form as presently being claimed.

(iii) Harris, *et al.* teaches away from the invention

The prior art must be considered as a whole, including portions that would lead one away from the claimed invention. *W.L. Gore & Assocs., v. Garlock, Inc.,* 721 F2d 1540 (Fed. Cir. 1983), *cert. denied* 469 U.S. 851 (1984). Harris, *et al.* teaches away from the present invention because Harris, *et al.* teach the necessity of P1' and P2' amino acids:

> [A]n optimal substrate for granzyme B was that spans over six subsites was determined to be Ile-Glu-Xaa-(Asp↓Xaa)-Gly, which cleavage of the Asp↓Xaa peptide bond. ***Granzyme B proteolysis was shown to be highly dependent on the length and sequence of a substrate.***

Abstract (emphasis added). Therefore, one of skill in the art would be led away from the present invention directed to a method wherein the Granzyme B cleavage site is "P4 P3 P2 P1 ↓", followed polypeptide of interest in authentic form. Instead, one of skill in the art following the teaching of Harris, *et al.,* and using six peptide cleave motif for which Harris, *et al.* teaches that proteolysis is ***highly dependent***, would be left with a polypeptide of interest with the two amino acids following the cleavage site, P1' and P2', and would not have a polypeptide of interest in authentic form as claimed.

(iv) The present invention provides unexpected results

Finally, claimed invention provides unexpected results. Compared to methods of preparing fusion proteins with other proteases known in the art, Granzyme B protease provides significant and unexpected improvement over the existing cited art. Granzyme B is (a) more specific than other proteases and avoids cleavages in the middle of the protein of interest, (b) permits the purification of authentic forms of proteins of interest with no extraneous amino acids at the amino terminus thereby improving native confirmation, and (c) provides a more efficient cleavage than other proteases, which reduces production costs by reducing wasted uncleaved fusion protein. Specification, pp. 3-5, and 62. Nothing in the cited art suggests to the skilled artisan that these goals can be accomplished using a Granzyme B protease as claimed.

In light of the arguments presented above, Applicants respectfully submit that Azad, *et al.* in view of Harris, *et al.*, and further in view of Casciola-Rosen, *et al.* do not render obvious independent claims 1 and 40, and dependent claims 9-11, 16, 17, 44-46, 50, and 51. Accordingly, Applicants respectfully request that the rejection of these claims under 35 USC § 103(a) be reversed.

B. Rejection of claims 4-6 and 41 under 35 U.S.C. 103(a) as obvious over Azad, *et al.*, Harris, *et al.*, and Casciola-Rosen, *et al.* in view of Boutin, *et al.*

Claims 4-6 depend, directly or ultimately, from independent claim 1; claim 41 depends from independent claim 40. For the reasons described above, claims 1 and 40 are not obvious over the combination of Azad, *et al.*, Harris, *et al.*, and Casciola-Rosen, *et al.* In addition, Applicants respectfully submit that Boutin, *et al.* does not add to the case of obviousness against claims 1 and 40. Accordingly, claims 4-6 and 41 are not obvious for the same reasons discussed above that claims 1 and 40 are not obvious.

In addition, the Examiner recognizes that Harris, *et al.,* Azad, *et al.,* and Casciola-Rosen, *et al.* does not "teach preparing a protein of interest by providing a fusion protein comprising, from the N-terminal to the C-terminal, a fusion partner, a Granzyme B cleavage motif, and the protein of interest followed by contacting the fusion protein with Granzyme B, wherein the polypeptide of interest is an enzyme." *See* Office Action mailed April 7, 2009, p. 8. To address this deficiency, the Examiner asserts that Boutin, *et al.* teaches that, like nef27 (*see* Azad, *et al.*), essentially all proteins that become myristoylated begin with Met-Gly at the N-terminus. The Examiner further asserts that Boutin, *et al.* teaches an enzyme, Calcineurin B, that begins with Met-Gly at the N-terminus (referring to Table 3). The Examiner points out that the N-terminal Met of Calcineurin B is removed co-translationally (referring to p. 16, paragraph 6 of Boutin, *et al.*) The Examiner concludes that it would have been obvious to a person skilled in the art to modify the fusion protein allegedly rendered obvious by the combination of Azad, *et al.* and of Harris, *et al.*, such that the nef27 protein is substituted with an enzyme, *i.e.,* calcineurin B, as taught by Boutin, *et al. Id.*

The Examiner's reasoning regarding the myristoylation of polypeptides and the removal of the N-terminal methionine is not relevant to the obviousness rejection. Even assuming that the Met was present, or that the protein is myristolyated, the Examiner's only reasoning for using Boutin, *et al.* in the rejection is that one of skill in the art would be motivated to produce Calcineurin B. *See* Office Action mailed April 7, 2009, p. 8. and Office Action mailed June 14, 2010, p. 13. Apparently, the only reason that Boutin, *et al.* is used in the rejection is related to the fact that Calcineurin is an enzyme that, assuming the Met is counted, would have a glycine that is penultimate to the N-terminus.

These reasons do not add to the case of obviousness based upon Harris, *et al.,* Azad, *et al.,* and Casciola-Rosen, *et al.*, alone, including with regard to dependent claims 4-6 and 41. With regard to claims independent 1 and 40, Boutin, *et al.* is not cited as teaching fusion proteins, any proteases cleaving fusion proteins, or the production of polypeptides using fusion proteins or proteases. Therefore, Boutin, *et al.* is irrelevant to claims 1 and 40. Therefore, the sole fact that Calcineurin B is an enzyme would not motivate one of skill in the art combine Boutin, *et al.* with Harris, *et al.,* Azad, *et al.,* or Casciola-Rosen, *et al.*

Therefore, Boutin *et al.* in combination with Harris, *et al.,* Azad, *et al.,* and Casciola-Rosen, *et al.* does not render obvious claims 4-6 and 41.

Accordingly, Applicants respectfully request that the rejection of claims 4-6, and 41 under 35 USC § 103(a) be reversed.

C. Rejection of claims 13-17 and 47-49 under 35 U.S.C. 103(a) as obvious over Azad, *et al.*, Harris, *et al.*, and Casciola-Rosen, *et al.*, in view of Sigma Inc. 1998 or Pharmacia, Inc.

The Examiner acknowledges that combination of Azad, *et al.*, Harris, *et al.*, and Casciola-Rosen, *et al.* does not teach a method wherein the Granzyme B is immobilized and cites Sigma Inc. 1998 and Pharmacia Inc. as teaching the use of immobilized proteases. Based on that, the Examiner concludes that it would have been obvious to one skilled in the art to modify the method allegedly rendered obvious by the combination of Azad, *et al.*, Harris, *et al.*, and Casciola-Rosen, *et al.* to used immobilized Granzyme B. The Examiner's conclusion is incorrect.

Sigma Inc. 1998 teaches a Thrombin CleanCleave™ Kit containing Sigma's thrombin-agarose suspension used to cleave fusion proteins containing a thrombin cleavage site. Sigma Inc. 1998 further teaches that the optimal cleavage sites for thrombin are: a) P4-P3-Pro-Arg/Lys • P1'-P2', wherein P4 and P3 are hydrophobic residues, P1' and P2' are non-acidic residues and Arg/Lys • P1'is the scissile bond and b) P2-Arg/Lys • P1' where P2 or P1' is glycine and Arg/Lys • P1' is the scissile bond. Pharmacia teaches sepharose coupling gels for the immobilization of ligands via a specific functional group. However, neither Sigma Inc. 1998's description of Thrombin-Agarose suspension nor Pharmacia Inc.'s description of sepharose coupling gels teach the production of a polypeptide in authentic form as presently claimed. Thus, the addition of neither Sigma Inc. 1998 nor Pharmacia Inc. cures the deficiency of the combination of Azad, *et al.*, Harris, *et al.*, and Casciola-Rosen, *et al.* to render obvious independent claims 1 and 40. Therefore, the combination can not render obvious dependent claims 13-17 and 47-49.

Accordingly, Applicants respectfully request that the rejection of claims 13-17 and 47-49 under 35 USC § 103(a) be reversed.

D. Rejection of claims 8 and 43 under 35 U.S.C. 103(a) as obvious over Wan, *et al.*, in view of Bleackley, *et al.*, and further in view of Harris, *et al.*

Applicants request that this rejection be reversed because the combination of Wan, *et al.*, Bleackley, *et al.*, and Harris, *et al.* is not asserted as rendering obvious independent claims 1 and 40, from which claims 8 and 43 depend. Because claims 1 and 40 are not obvious for the reasons stated above, depend claims 8 and 43 are also not obvious.

VII. CONCLUSION

Applicants respectfully request that all of the rejections of the pending claims be reversed.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff LLP

Date: February 14, 2011

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CLAIMS APPENDIX

1. A method for the preparation of a polypeptide of interest in authentic form, said method comprising the steps of:

(i) providing a fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site that is cleavable by human Granzyme B protease, and wherein the recognition site comprises an amino acid sequence of the general formula

P4 P3 P2 P1 ↓ (SEQ ID NO: 59)

wherein

P4 is amino acid I or V,

P3 is amino acid E, Q or M,

P2 is X, where X denotes any amino acid,

P1 is amino acid D, and

↓ is said Granzyme B protease cleavage site, and

(c) the polypeptide of interest, wherein said cleavage site is adjacent to the polypeptide of interest, and

(ii) cleaving the fusion protein with Granzyme B protease at said cleavage site to yield said polypeptide of interest in authentic form.

4. The method according to claim 1 wherein the N-terminus of the polypeptide of interest is adjacent to the cleavage site and the penultimate amino acid at the N-terminus of the polypeptide of interest is glycine.

6. The method according to claim 1, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.

8. The method according to claim 6, wherein the enzyme is Granzyme B.

9. The method according to claim 1, wherein the fusion partner is an affinity-tag.

10. The method according to claim 9, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.

11. The method according to claim 1, wherein the fusion protein is cleaved with a Granzyme B protease selected from the group consisting of human Granzyme B protease, mouse Granzyme B protease and rat Granzyme B protease.

13. The method according to claim 1, wherein the Granzyme B protease is in an immobilised form.

14. The method according to claim 13, wherein the Granzyme B protease is immobilised via the C-terminus.

15. The method according to claim 13, wherein the Granzyme B protease is immobilised via a lysine amino acid residue.

16. The method according to claim 10, wherein the affinity-tag is a polyhistidine-tag, and wherein the fusion protein is contacted with said Granzyme B protease in the presence of Ni^{2+} ions and Nitrilotriacetic Acid (NTA).

17. The method according to claim 16, wherein the concentration of Ni^{2+} is in the range of 1-20 mM, and the concentration of NTA is in the range of 1-20 mM.

40. A method for the preparation of a polypeptide of interest in authentic form, said method comprising the steps of:

    a. providing a fusion protein comprising, from its N-terminal to its C-terminal, (a) a fusion partner, (b) a Granzyme B protease recognition site comprising a Granzyme B protease cleavage site that is cleavable by human Granzyme B, wherein the recognition site comprises an amino acid sequence selected from the group consisting of ICPD↓ (SEQ ID NO: 61), IEAD↓(SEQ ID NO: 62), IEPD↓(-SEQ ID NO: 63), IETD↓(SEQ ID NO: 64), IQAD↓(SEQ ID NO: 65), ISAD↓(-SEQ ID NO: 66), ISSD↓(SEQ ID NO: 67), ITPD↓(SEQ ID NO: 68), VAPD↓(-

SEQ ID NO: 69), VATD↓(SEQ ID NO: 70), VCTD↓(SEQ ID NO: 71), VDPD↓(-SEQ ID NO: 72), VDSD↓(SEQ ID NO: 73), VEKD↓(SEQ ID NO: 74), VEQD↓(-SEQ ID NO: 75), VGPD↓(SEQ ID NO: 76), VEID↓(SEQ ID NO: 77), VRPD↓(-SEQ ID NO: 78), VTPD↓(SEQ ID NO: 79), LEED↓(SEQ ID NO: 80), LEID↓(-SEQ ID NO: 81), LGND↓(SEQ ID NO: 82), LGPD↓(SEQ ID NO: 83), and AQPD↓(SEQ ID NO: 84), and wherein ↓ is said Granzyme B protease cleavage site, and the polypeptide of interest, wherein said cleavage site is adjacent to the polypeptide of interest, and

b. cleaving the fusion protein with Granzyme B protease at said cleavage site to yield said polypeptide of interest in authentic form.

41. The method according to claim 40, wherein the polypeptide of interest is selected from the group consisting of an enzyme, a polypeptide hormone, a single chain antibody variable region fragment, and apolipoprotein A.

43. The method according to claim 41, wherein the enzyme is Granzyme B.

44. The method according to claim 40, wherein the fusion partner is an affinity-tag.

45. The method according to claim 44, wherein the affinity-tag is selected from the group consisting of a polyhistidine-tag, a polyarginine-tag, a FLAG-tag, a Strep-tag, a c-myc-tag, a S-tag, a calmodulin-binding peptide, a cellulose-binding peptide, a chitin-binding domain, a glutathione S-transferase-tag, and a maltose binding protein.

46. The method according to claim 40, wherein the fusion protein is cleaved with a Granzyme B protease selected from the group consisting of human Granzyme B protease, mouse Granzyme B protease and rat Granzyme B protease.

47. The method according to claim 40, wherein the Granzyme B protease is in an immobilised form.

48. The method according to claim 47, wherein the Granzyme B protease is immobilised via the C-terminus.

49. The method according to claim 47, wherein the Granzyme B protease is immobilised via a lysine amino acid residue.

50. The method according to claim 44, wherein the affinity-tag is a polyhistidine-tag, and wherein the fusion protein is contacted with said Granzyme B protease in the presence of Ni^{2+} ions and Nitrilotriacetic Acid (NTA).

51. The method according to claim 50, wherein the concentration of Ni^{2+} is in the range of 1-20 mM, and the concentration of NTA is in the range of 1-20 mM.

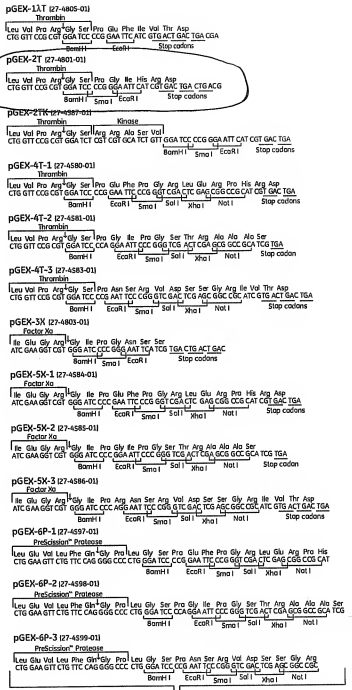
EVIDENCE APPENDIX

Ex. A. – map of pGEX-2T vector filed by Applicants as Ex. A to Response to Office Action on June 8, 2009 and entered by the Examiner with the Advisory Action mailed June 18, 2009.

pGEX Vectors, GST Gene Fusion System

Map of the glutathione S-transferase fusion vectors showing the reading frames and main features. Even though stop codons in all three frames are not depicted in this map, all thirteen vectors have stop codons in all three frames downstream from the multiple cloning site.

Do you want to learn more? Read the GST Gene Fusion System Handbook (18-1157-58). Please contact your local GE Healthcare representative for a printed copy.



For contact information for your local office, please visit, www.gelifesciences.com/contact

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RELATED PROCEEDINGS APPENDIX

None